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(54) Title: TARGETED ANTIOXIDANTS

(57) Abstract

Compounds which consist of an antioxidant moiety chemically linked to the precursor moiety which is capable of targeting specific populations of cells by exploiting endogenous uptake systems for biological chemicals are useful in the treatment of neurodegenerative disorders including Parkinson's disease, Alzheimer's disease and neuropathological conditions induced by amphetamines such as MDMA.

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TARGETED ANTIOXIDANTS

The present invention relates to antioxidants for use in medicine and clinical research. In particular, the invention relates to antioxidants which are targeted to particular areas of the body and, especially, the brain and central nervous system.

Reactive oxygen species are constantly formed in biological systems but, normally, free radical production and consequent tissue damage are controlled by a series of naturally occurring antioxidant defence mechanisms. Oxidative phosphorylation primarily takes place in mitochondria where reactive oxidant species are tightly bound and can be safely reduced to water. The superoxide radical is dismutated by superoxide dismutase to hydrogen peroxide which can then be removed by either catalase or glutathione peroxidase. Chain breaking antioxidants or free radical scavengers such as α-tocopherol (vitamin E) can react directly with free radicals and thereby spare more critical molecules.

Because molecular oxygen reacts readily with transition metals such as iron or copper, oxidation reactions can be influenced by the regional concentration of a transition metal. Recycling of iron from its oxidised state to its reduced state by tissue ascorbate, glutathione or dopamine can drive oxidation reactions and the formation of a cascade of free radicals. Iron is more likely to participate in redox reactions when it is in a low oxidation state, usually complexed to ATP or citrate. Proteins such as transferrin or ferritin which bind iron and maintain it in a relatively non-reactive state thus serve as another important antioxidant defence mechanism.

Normally, there is an equilibrium between factors that promote free radical formation and the antioxidant defence mechanisms. An imbalance in this equilibrium which favours the formation of free radicals is known as oxidative stress.

Oxidative stress is associated with many medical conditions because of the tissue damage caused by free radicals. For example, the superoxide radical may react with endogenous nitrogen monoxide to give peroxynitrite which can then be protonated to form the nitrosyl radical which decomposes to give the hydroxyl radical. Peroxynitrite is a highly reactive oxidising agent capable of causing tissue damage and the reaction between superoxide and nitrogen monoxide has been proposed to mediate toxicity associated with excitatory amino acids and with ischemia and reperfusion and tissue damage and can be blocked by agents that inhibit superoxide or nitrogen monoxide formation.

In models of excitotoxicity as well as in a number of neurodegenerative disorders, including Huntington's disease, Alzheimer's disease and Parkinson's disease, free radicals are associated with, and promote, tissue damage.

Brain cells appear to be at particular risk from free radical damage. The mammalian brain contains large amounts of substrates, such as unsaturated lipids and catecholamines, which are susceptible to free radical attack. Polyunsaturated fatty acids are a major constituent of cell membranes and a substrate for lipid peroxidation. In addition, iron, which promotes cytotoxic radical formation, accumulates in specific brain regions, such as the globus pallidus and the substantia nigra, in concentrations which exceed those found in the liver. Defence mechanisms in the brain are relatively deficient as the brain contains almost no catalase and has reduced concentrations of glutathione, glutathione peroxidase and vitamin E. Iron binding capacity appears to be limited in the CNS. Therefore, any imbalance of cellular redox status in favour of greater oxidative activity can lead to several kinds of macromolecular damage such as disruption of genomic function by alterations to DNA or impairment of membrane properties by attack on proteins and lipids. Lipid peroxidative events are especially

hazardous since lipoperoxy radicals can initiate chain reactions. Thus, the high lipid content of myelin makes nervous tissue especially susceptible to oxidative stress.

Reactive oxygen species are implicated in the pathogenesis of a wide variety of human and animal diseases. Thus, antioxidants may be of clinical use in the treatment of several types of diseases and conditions, in particular, diseases and conditions associated with oxidative damage to brain tissue.

A clinical trial known as DATATOP (deprenyl and tocopherol antioxidative therapy of Parkinsonism) was initiated to test whether deprenyl (selegiline), a monoamine oxidase-B inhibitor which should prevent metabolism of dopamine and maintain dopamine levels and also prevent the formation of free radicals and/ or vitamin E, a free radical scavenger, could slow or halt the progression of Parkinson's disease (Scoulson *Eup. Neurol.*, (1992) **32**, 46-53, Lewitt *Eup. Neurol.*, (1993) **33**, 24-30 and Schneider *J. Neural Trans.*, (1995) **suppl. S46**, 391-397).

While the results are not conclusive, coadministration of drugs(10 mg deprenyl +/- 2000 mg vitamin E per day) appeared to delay the onset of disability and the requirement for levodopa therapy. This supports the proposal that antioxidant drug therapy may arrest the progression of Parkinson's disease. Treatment with deprenyl blocks the metabolism of dopamine by monoamine oxidase and thus prevents the formation of hydrogen peroxide which is a product of this reaction.

The data from the DATATOP study showed, however, that vitamin E therapy alone afforded no neuroprotection and it seems that the reason for this may be that the dosages employed did not reach therapeutically efficacious brain levels. It therefore appears that targeting of vitamin E to the neuronal population may be a significant problem which prevents vitamin E from effectively combating Parkinson's disease.

Another area in which antioxidants have been used therapeutically was in an attempt to design targeted chemotherapy (antitumour) agents for disseminated neuroblastoma, a type of neural cancer (Purpura *et al, Cancer Res.*, (1996) **56**, 2336-2442). In this study, the dopamine uptake system on neuroblastoma cells was used to target the cells. 6-Hydroxydopamine (6-OHDA), a dopamine neurotransmitter analogue generates cytolytic oxygen radicals in neuroblastoma cells that take it up. This analogue is, however, systemically toxic due to its spontaneous oxidation; this toxicity is particularly severe in the sympathetic nervous system since such tissues selectively concentrate dopamine and its analogues. Lowering the dose of 6-OHDA below systemic toxic levels prohibitively compromises its antitumour effect. To avoid both the systemic and the sympathetic nervous system toxicity, the group administered an antioxidant (Tempol, 250 mg/kg, i.p.) prior to giving 6-OHDA. This combination of pretreatment with an antioxidant followed by treatment with 6-OHDA reduced the cancer mortality and systemic toxic effects due to 6-OHDA.

Lazaroids are inhibitors of free radical formation and lipid peroxidation. The effects of two lazaroids, U-74389G (21-aminosteroid) and U-83836E (combines the piperazinyl pyrimidine portion of 21-aminosteriod with the antioxidant ring of α-tocopherol) were studied on the survival of cultured rat dopamine neurons (Frodl *et al, NeuroReport*, (1994), **5**, 2393-2396 and Nakao *et al, Proc, Natl. Acad. Sci. USA*, (1994) **91**, 12408-12412). The lazaroids were shown to enhance the survival of dopaminergic neurons *in vitro* but the total number of neurons was also increased by lazaroids and therefore the lazaroids did not specifically target the dopaminergic neurons. This study indicates that reduction of free radical formation is effective in reducing neuronal loss relevant to the pathogenesis of neural disease such as Parkinson's disease.

Antioxidants such as vitamin E are also used as dietary supplements and have been shown to ameliorate renal injury in an experimental model of immune-mediated nephropathy (Trachtman *et al*, *Paed. Res.*, (1996), **40**, 620-626) and supported the use of dietary treatment with this antioxidant in patients with similar disease. Vitamin E supplementation was also shown to reduce iron-induced oxidative stress in the liver (Parkkila *et al*, *Am. J. Physiol - GI & Liver Physiol.*, (1996), **33**, 376-384). High dose vitamin E may also slow the rate of motor function decline in the course of Huntington's disease.

Thus, it has been suspected for some time that antioxidants could be useful agents for the treatment of various diseases and conditions, particularly neurological diseases or conditions but also other conditions such as renal disease. However, there have been problems with the use of antioxidants in the treatment of such conditions, the main problem being that it is difficult to target the antioxidant to the required tissue. This problem is particularly acute for diseases of the central nervous system.

In order to overcome the problems of correctly targeting the antioxidant, the present inventors have devised a system which exploits endogenous uptake systems for biological chemicals.

Therefore, in a first aspect of the present invention, there is provided a compound comprising an antioxidant moiety chemically joined to a targeting moiety capable of targeting specific populations of cells by exploiting endogenous uptake systems for biological chemicals.

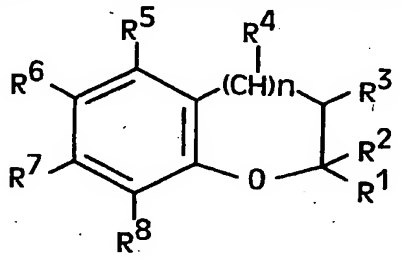
The advantage of such compounds is two fold. Firstly, the antioxidant is targeted to the particular cells where it is required and, secondly, the targeting moiety will

metabolise to form a compound which will replenish biological cellular chemicals important in homeostasis.

The joining of an antioxidant moiety with a targeting moiety to form a targeted antioxidant permits targeted delivery of the antioxidant directly to specific groups of cells, including neurons, where cellular stress is associated with pathology, including that associated with normal ageing and neurodegenerative disorders. While antioxidant therapy has been explored for therapeutic efficacy in a number of pathological conditions, joining the antioxidant to a group capable of targeting a specific population of dying cells has not previously been considered.

The antioxidant moiety may be either an enzymatic or a non-enzymatic antioxidant which possesses the ability to reduce the formation and/or production of free radical species generated by redox reactions of oxygen and nitrogen occurring in biological systems. Many such antioxidants are known and they include α-tocopherol (vitamin E), other tocopherols, β-carotene, ascorbic acid (vitamin C) and many others.

Analogues of α-tocopherol may also be used as antioxidants and suitable antioxidants based on α-tocopherol and its analogues are represented by general formula I:



(I)

wherein:

R^1 is C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, aryl or heteroaryl, any of which may optionally be substituted with one or more fluoro, chloro, bromo, amino, nitro, azo or diazo groups.

Each of R^2, R^4, R^5, R^7 and R^8 is, independently, hydrogen, fluoro, chloro, bromo or C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, any of which may be substituted with one or more fluoro, chloro, bromo, amino, nitro, azo or diazo groups;

R^3 is hydrogen or, together with R^2 or R^4, may form a double bond;

R^6 is OH, NHR^9,SH, Cl, Br or F;

R^9 is C_1-C_6 alkyl, C_2-C_6 alkenyl or C_2-C_6 alkynyl;

n is 0 or 1.

In the context of the present invention, the term C_1-C_6 alkyl refers to a straight or branched alkyl group having from one to six carbon atoms. Examples of such groups include methyl, ethyl, n-propyl, isobutyl and tertiary butyl. References to C_1-C_8 alkyl and C_1-C_{24} alkyl should be construed accordingly.

The term C_2-C_6 alkenyl refers to a straight or branched alkenyl group having from two to six carbon atoms and one or more carbon-carbon double bonds. Examples of such groups include ethenyl, propenyl, 1-butenyl, 2-butenyl and isoprenyl. References to C_2-C_8 alkenyl and C_2-C_{24} alkenyl should be construed accordingly.

The term C_2-C_6 alkynyl refers to a straight or branched alkynyl group having from two to six carbon atoms and one or more carbon-carbon triple bonds. Examples of such

groups include ethynyl, propynyl, 1-butynyl and 2-butynyl. References to C_2-C_8 alkynyl and C_2-C_{24} alkynyl should be construed accordingly.

The term aryl refers to an aromatic carbocyclic ring system having from five to 14 carbon atoms and one or more aromatic rings. Examples of such systems include phenyl, naphthalenyl and anthracenyl. The aryl group may be partially saturated, for example tetrahydronaphthalenyl, provided that it retains aromatic character.

The term heteroaryl refers to an aromatic ring system having one or more aromatic rings and from 5 to 14 atoms, at least one of which is a heteroatom selected from nitrogen, oxygen or sulphur. As for the aryl groups, the hetroaryl group many be partially saturated, provided that it retains aromatic character. Examples of heteroaryl groups include pyridyl, pyrimidyl, furanyl, pyranyl, benzofuranyl, imidazolyl, imidazolinyl and benzimidazolinyl.

Preferred compounds include those in which R^6 is OH or NH_2 and R^1 is C_2-C_{24} alkenyl optionally substituted by one or more azo or diazo groups, R^5, R^7 and R^8 are methyl or ethyl and n is 1, and fluorinated derivatives of such compounds.

Particularly preferred compounds are α-tocopherol itself (R^1 is OH R^2 is phytyl, R^3, R^4 and R^5 are methyl and n is 1), β-tocopherol and their derivatives in which the phytyl group is substituted by one or more azo or diazo groups.

Compounds of formula I are particularly suitable for inclusion in compounds which are intended to treat disorders of the central nervous system.

Examples of specific antioxidants which can be used in the compounds of the present invention include:

β-tocopherol;

2,2,5,7,8-pentamethylbenzopyran;

6-chloro-2,2,-dimethylbenzopyran;

7,8-dihydroxy-2,2-dimethylbenzopyran;

1,2-dihydro-3,3-dimethyl-3H-naphtho-[2,1-b]-pyran;

6-hydroxy-2,2,-dimethylbenzopyran;

5-hydroxy-2,2,4,6,7-pentamethylbenzofuran;

2,2-dimethylbenzofuran;

6-hydroxy-2-methoxy-2,5,7,8-trimethylbenzopyran

6-hydroxy-2,2,4,5,7,8-hexamethylbenzopyran

2,3-Dihydro-6-hydroxy-2,5,7,8,-tetramethyl-2-(tridecyl) benzopyran;

2,3-Dihydro-6-hydroxy-2,5,-dimethyl-2-(4',8',12'-trimethyltridecyl) Naphthopyran

2,3-Dihydro-6-hydroxy 2,2,5,7,8-pentamethylbenzopyran

2,3-Dihydro-6-hydroxy 2,2,5,7,-tetramethylbenzopyran

2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethylbenzofuran

2-Methoxy-2,5,7, 8,teramethylchroman-6-ol

2,6-Dihydroxy-2,5,7,8-tetramethylbenzopyran

2-ene-6-hydroxy -2,5,7,8-tetramethylbenzopyran

6-hydroxy-5,7,8-trimethyl-2-oxo-benzopyran-3-carboxylic acid.

Other antioxidants which may be coupled to targeting moieties to form compounds of the present invention include antioxidants such as vitamin C and protein antioxidants such as superoxide dismutase, catalase and glutathione peroxidase.

In addition, the antioxidant may be a compound which can trap oxidising radicals at low tension environments. Such compounds include carotene-like substances, for example retinoic acid.

In the context of the present invention, a targeting moiety may be a group which is the precursor of a biological cellular chemical and which can be taken up by specific populations of cells, including neurons, *via* various routes, for example diffusion, facilitated diffusion, carrier systems and active processes.

Alternatively, the targeting moiety may be a transport system amino acid.

Examples of targeting moieties include amine precursors such as levodopa, 5-hydroxytryptophan, choline and histidine, which are the precursors respectively of dopamine, 5-hydroxytryptamine, acetylcholine and histamine; polyamine precursors such as spermine, spermidine and putrescine and amino acids such as glutamate and aspartate, which are transport systems for glutamatergic and gabaergic cells respectively.

In some cases, a protected version of the targeting moiety may be used and this is particularly useful for precursors such as levodopa, which is sparingly soluble in organic solvents making it difficult to prepare the compounds of the present invention.

Suitable protected targeting groups include boc, neuropeptides, peptides or α-alkylated amino acid peptides (peptoids). These protected forms of the targeting moiety can be chemically joined to the anti-oxidant with or without an intervening link in the molecule of the present invention and administered to a patient as prodrugs. In the case of levodopa, it is advantageous to protect both the phenolic and the carboxylic portions of the molecule to prevent premature oxidation of such groups.

The targeting moiety and the anti-oxidant may be chemically joined by means of a covalent bond. However, superior results have been obtained when the anti-oxidant

and the targeting moiety are joined *via* a linking group, which preferably contains nitrogen.

Examples of linker groups include -NH-, methylene chains of from 1 to 50 carbon atoms and optionally containing one or more hetero atoms, which may either interrupt the methylene chain or be at one or both ends of the chain. Shorter linkers of from 1 to 20 carbon atoms may, however, be preferred and it is also preferred that the linker is formed from a diamine and, thus, has an -NH- group at either end of the chain. Examples of linkers of this type include ethane-1,2-diamine; propane-1,2-diamine; racemic, RR and SS forms of cyclohexane 1,2-diamine; propane 1,3-diamine; butane-1,4-diamine, pentane-1,5-diamine; 2-methylpentane-1,5-diamine; hexane-1,6-diamine; heptane-1,7-diamine; octane-1,8-diamine; nonane-1,9-diamine; decane -1,10-diamine; dodecane-1,12-diamine; putrescine, cadavarine, spermine and spermidine.

Alternatively, the linker group may be an aromatic, heteroaromatic, carbocyclic or alicyclic ring having from 5 to 14 atoms and one or more rings. Such ring systems may link the antioxidant and the targeting moiety *via* nitrogen atoms, for example in diaminocyclohexyl and 1,3-diaminoadamantyl.

The compounds of the present invention may be prepared in a variety of ways depending upon the nature of the anti-oxidant, the precursor and the chemical linkage.

Anti-oxidants of general formula I may be prepared by methods known to those skilled in the art. For example, a phenol or hydroquinone derivative may be reacted with isoprene or a propenol or butenol derivative. Greater details of such syntheses are given in Example 1 below and in the literature, for example *Vitamin E: A Comprehensive Treatise*, L J Machlin (Ed), Marcel Dekker, New York (1980), L J Machlin in *Handbook of Vitamins, Nutritional, Biochemical and Clinical Aspects*,

Chapter 3, L J Machlin (Ed) Marcel Dekker, New York (1984) pages 7-65 and Ismail *et al Tetrahedron Lett.* **33(26)** 3795-3796 (1992).

In the case where the linkage is a chemical bond, the anti-oxidant and the targeting moiety may be coupled by any suitable technique which would be familiar to one skilled in the art of chemistry.

When either the targeting moiety or the anti-oxidant contains a free amine group, the following method can be used. The same method is appropriate for coupling an antioxidant moiety to a linker group (which may already have been joined to a targeting moiety) and in which either the antioxidant moiety or the linker contains a free terminal amine group. In the description below, the amine group is referred to as being on the targeting moiety or linker but the method would be equally effective when the free amine group is on the anti-oxidant.

Firstly, a halogenated derivative of the anti-oxidant moiety is prepared. The halogenated derivative is preferably a brominated or a chlorinated derivative and may be prepared using any conventional method. Brominated derivatives of antioxidants of general formula I may be synthesised by reacting the compound of general formula I with one mole equivalent of bromine in a dry solvent under an atmosphere of either nitrogen or, preferably, argon. Bromine preferentially attacks at allylic positions within the molecule. The reaction may be conducted in the presence or absence of UV light, which can influence the site of bromination. The particular effects of UV light in such reactions will be familiar to those skilled in the art.

Examples of suitable reaction solvents include chloroform, dichloromethane and carbon tetrachloride. The reaction can also be conducted in suitable hydrocarbon solvents and is only limited by the solubility of the compounds that require bromination.

Fluorocarbon solvents can also be used provided the reaction is cooled to ensure that the solvent remains liquid. The cooling may be carried out by any of a variety of techniques known to those skilled in the art. The preferred reaction solvent is carbon tetrachloride.

The reaction can also be conducted using N-methylpyrrolidin-2-one. If desired, the reaction may be assisted by use of an acid scavenger such as sodium hydrogen carbonate or, preferably, triethylamine. Any other suitable tertiary amine can be used, provided that it is not unsaturated, for example trimethylamine, tripropylamine, etc.

The reaction is performed with external cooling and must be below 0°C during the addition of bromine.

Under low temperature, dark conditions, methyl substituents in the 5-position of benzopyrans are preferentially attacked and those in the 7-position less so. The reaction products may be separated from one another using gravity column, flash column medium or high pressure liquid chromatography and capillary zone electrophoresis. The preferred material for separation is either silica or alumina. Higher molecular weight compounds are preferably separated using medium pressure liquid chromatography or gel exclusion/permeation chromatography.

Next, the halogenated anti-oxidant is reacted in solution with the targeting moiety or linker which, as mentioned above, contains a free amine group. Suitable solvents include halogenated solvents such as carbon tetrachloride, chloroform or dichloromethane or other non-polar or polar aprotic solvents such as diethyl ether, n-hexane, petroleum ether, dioxane or tetrahydrofuran. The reaction temperature may be from -50 to 10°C, preferably from about -30 to -5°C and typically about -25 to -20°C.

It is preferred that the reaction is carried out under an inert atmosphere such as nitrogen or argon.

The product of this reaction is an ammonium halide derivative and this may be converted to the required compound by reaction with a base, typically a strong base such as an alkali metal hydroxide, typically sodium hydroxide. The reaction may be carried out in an organic solvent such as dichloromethane or, alternatively, in aqueous solution if the ammonium halide is insoluble or sparingly soluble in organic solvents.

This method is particularly useful for the coupling of tocopherols and other anti-oxidants of general formula I to targeting moieties and linkers such as levodopa, 5-HT, choline, histidine, spermine, spermidine, putrescine, glutamate and aspartate.

If a linker is used in this method, it may be coupled to the targeting moiety either before, after, or simultaneously with the coupling reaction to the anti-oxidant. In coupling the linker to the targeting moiety, standard methods may be used, for example, a linker with a terminal amino group may be coupled to a targeting moiety with a halo or ester group or a linker with a terminal ester or halo group may be coupled to a targeting moiety with a free amino group using standard methods known to those skilled in the art. When the linker is symmetrical and has two terminal amino groups and the targeting moiety is halogenated, the coupling reactions of the linker to the targeting moiety and the anti-oxidant can be carried out simultaneously. This method is appropriate for both chain and cyclic linkers.

Thus, the above method may be used in the following ways:

a) reacting a brominated antioxidant with a targeting moiety having a free amine group;

b) reacting a halogenated antioxidant with a targeting moiety attached to a linker having a free amine group;

c) reacting a halogenated antioxidant and a halogenated targeting moiety simultaneously with a linker having two free amine groups;

d) reacting a halogenated targeting moiety with an antioxidant having a free amine group; or

e) reacting a targeting moiety attached to a halogenated linker with an antioxidant having a free amine group.

In some cases the targeting moiety may be reacted with an anti-oxidant precursor so that an antioxidant moiety is formed during the course of the reaction. Thus, the reaction of an amine-containing targeting moiety such as dopa with trimethyl tetrahydroquinone and formaldehyde leads to the formation of a 1,3-oxazoline ring system in which the nitrogen atom is the nitrogen atom of dopamine. The reaction may be conducted simply by heating the reactants under reflux in an appropriate solvent such as N-methyl pyrrolidone or a mixture of dimethylsulfoxide and dimethylformamide.

The novel compounds of the present invention are useful in a variety of therapeutic indications and therefore in a further aspect of the present invention, there is provided a compound comprising an antioxidant moiety chemically joined to a precursor chemical moiety capable of targeting specific populations of cells by exploiting endogenous uptake systems for biological chemicals for use in medicine.

Because of its particular usefulness in targeting cells of the central nervous system, especially neurons, the present invention is of use in the treatment of neurodegenerative disorders, including those associated with aging.

The central nervous system (CNS) is particularly susceptible to the cytotoxic activities of free radical attack because of its high lipid content, high rate of oxidative metabolism

and relatively low levels of endogenous free radical scavenging systems. In addition, various areas of the CNS are rich in iron, for example the substantia nigra and corpus striatum. For this reason, the invention is particularly useful for targeting cells of the CNS.

As briefly mentioned above, when the target cells are in the CNS, α-tocopherol and its variants of formula I have been found to be particularly suitable antioxidants. The reason for this is that α-tocopherol has been found to be protective against chemically induced neurotoxicity (Bondy. *NeuroToxicol.* (1992) **13** 87-100). The primary biological function of α-tocopherol is the protection of polyunsaturated fats in membranes against oxidation. It is incorporated into cell membranes and thereby protects against free-radical attack in the lipid phase. In conditions of oxidative stress such are found in many CNS disorders, brain concentrations of vitamin E and other antioxidants are reduced. Provision of the vitamin E or other antioxidants to the specific neuronal population undergoing oxidative stress by joining the antioxidant to a suitable targeting moiety, such as an amine, enables targeted delivery of the antioxidant to the neurone population under stress.

The majority of endogenous antioxidant systems in the CNS are present in neural cells other than neurons. This means that neurons are particularly vulnerable to the effects of oxidative stress. Therefore, it is especially preferred that, in the present invention, the cells which are targeted by the targeting moiety are neurons, for example dopaminergic, serotoninergic, cholinergic or histaminergic neurons.

For these cells, the targeting moiety is preferably an amine required for the production of neurotransmitter chemicals. For the examples of neurons given above, the targeting moiety may be levodopa, 5-hydroxytryptophan, choline or histidine, the precursors respectively of the neurotransmitters dopamine, serotonin (5-hydroxytryptamine),

acetylcholine and histamine. The precursor amine moiety accesses specific nerve terminals by exploiting uptake systems present on neuronal membranes and thus makes possible the targeted delivery of the antioxidant moiety directly to specific groups of cells.

When the targeted cell is a dopaminergic receptor and the targeting moiety is levodopa or a protected form thereof, the compound of the present invention may be used in a method for the treatment of Parkinson's disease, the method comprising administering to a patient an appropriate amount of a compound comprising an antioxidant chemically joined to levodopa. In this case, the antioxidant is preferably α-tocopherol or another compound of general formula I, although other antioxidants may also be employed.

Parkinson's disease is also known as striatal dopamine deficiency and involves the destruction of the dopamine neurons of the nigrostriatal pathway. Dopaminergic circuitry is especially vulnerable to free radical damage and progression of the disease is associated with free radical attack leading to further loss of dopamine neurons.

In the early stages of Parkinson's disease, there appears to be a compensatory increase in the number of dopamine receptors to accommodate the initial loss of dopamine neurons. As the disease progresses, however, the number of dopamine receptors decreases, apparently due to the concomitant degeneration of dopamine target sites on striatal neurons. The loss of dopaminergic neurons in Parkinson's disease results in enhanced metabolism of dopamine, augmenting the formation of highly neurotoxic hydroxyl radicals. Studies of the substantia nigra after the death of patients with Parkinson's disease have suggested the presence of oxidative stress and depletion of reduced glutathione, a high level of total iron with reduced levels of ferritin and a deficiency of mitochondrial complex I; all events associated with oxidative stress.

Free radicals have been implicated in Parkinson's disease because:

i. dopamine turnover is likely to be increased in surviving neurons with a resultant increase in the formation of hydrogen peroxide;

ii. levels of reduced and total glutathione are decreased in the substantia nigra suggesting decreased protection against hydroxyl radical formation;

iii. iron concentration is increased in the substantia nigra thereby increasing the likelihood of an interaction with hydrogen peroxide and consequent formation of the hydroxyl radical;

iv. lipid peroxidation is increased in the substantia nigra.

These findings indicate that in Parkinson's disease the substantia nigra is in a state of oxidative stress. Further, a decrease in reduced glutathione has been found in patients presumed to have preclinical Parkinson's disease at autopsy. This suggests that oxidative stress may be present at the earliest stages of the disorder and that a deficiency in scavenger antioxidants could be crucial to the progression of Parkinson's disease.

The compounds of the present invention in which levodopa or a protected form thereof is chemically joined to an antioxidant such as vitamin E exploit the dopamine uptake system of dopaminergic neurons and thereby deliver a non-enzymic antioxidant directly to the stressed neurons together with the precursor required in the manufacture of dopamine to restore neurotransmitter levels.

Therefore, the invention provides, in a further aspect, a compound comprising an antioxidant, preferably a compound of general formula I, chemically joined to levodopa or a protected form thereof for use in the treatment of Parkinson's disease.

The invention also provides the use of a compound comprising an antioxidant, preferably a compound of general formula I, chemically joined to levodopa or a protected form thereof, in the preparation of an agent for the treatment of Parkinson's disease.

A second clinical use for the compounds of the present invention is in a method for the treatment of substituted amphetamine neuropathology arising from the recreational use of drugs such as 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy), the method comprising administering to a patient a compound comprising levodopa or a protected form thereof and/or 5-hydroxytryptophan chemically joined to an antioxidant, for example a compound of general formula I.

An increasing number of reports document the severe acute toxicity arising from abuse of MDMA and there is growing concern that it may cause long term toxic damage to serotoninergic neurons; dopaminergic pathways may also be affected. The neurotoxicity of MDMA may in part be due to oxidative stress relating to dopaminergic and serotoninergic circuitry, inferred by attenuation of induced neuropathological changes by pretreatment with a variety of antioxidants.

There is increasing concern about the potential short- and long-term consequences of MDMA ingestion. Various experimental studies with rodents and non-human primates have demonstrated neurotoxic effects due to a central depletion of 5-HT and a selective degeneration of serotoninergic neurons after administration of MDMA in dosages that

are comparable with those used by humans. The neuropsychiatric risk of MDMA is difficult to assess but should not be underestimated.

Although the large majority of individuals who have used MDMA recreationally do not develop acute complications, as the popularity of MDMA has increased, so have reports of adverse non-psychiatric and psychiatric consequences associated with the use of the drug. Further, since manifestations of MDMA-induced 5-HT injury might only become apparent with age or under periods of stress, it is possible that some individuals with no apparent abnormalities may develop complications over time.

Experiments in awake behaving laboratory animals have shown that single injections of MDMA increase extracellular levels of dopamine and 5-HT in the nucleus acumbens and in several other brain regions that are important for reward. Most of the behavioural and electrophysiological changes that have been reported to date for single doses of MDMA appear to be mediated by this increase in dopamine and 5-HT.

In view of this, a targeted antioxidant drug of the present invention comprising an antioxidant linked to 5-hydroxytryptophan and/or levodopa or a protected form thereof will be of use in the treatment of conditions arising from the use of MDMA and similar drugs.

Therefore, the invention provides, in a further aspect, a compound comprising an antioxidant, preferably a compound of general formula I, chemically joined to levodopa or a protected form thereof and/or 5-hydroxytryptophan for use in the treatment of neuropathological conditions induced by amphetamines such as MDMA.

The invention also provides the use of a compound comprising an antioxidant, preferably a compound of general formula I chemically joined to levodopa or a

protected form thereof and/or 5-hydroxytryptophan in the preparation of an agent for the treatment of neuropathological conditions induced by amphetamines such as MDMA.

A third clinical use for the compounds of the present invention is in a method for the treatment of Alzheimer's disease, the method comprising administering to a patient an effective amount of a compound according to the present invention and comprising an antioxidant such as a compound of general formula I chemically joined to choline and/or histidine.

Alzheimer's disease is the most common cause of progressive intellectual failure. The lesions that develop in the brain, called senile plaques, are extracellular deposits principally composed of insoluble aggregates of β-amyloid protein. β-Amyloid has been shown to exert a direct toxic effect on neurons *in vitro via* induction of oxidant stress; the antioxidant vitamin E prevents such toxicity. Alzheimer's disease is largely an age-related condition with an incidence that closely parallels increases in cellular oxidative stress and subsequent formation of dysfunctional proteins. Neuronal injury in Alzheimer's disease is associated with oxidant stress.

Alzheimer's disease is a multisystem disorder in which cholinergic and monoaminergic (especially histamine neuroimmune interactions) neurotransmissions are altered. The main focus of current therapeutic approaches is the maintenance of acetylcholine levels since basal forebrain cholinergic neurons are lost in Alzheimer's disease. However, in the limited number of patients who respond to acetylcholinesterase inhibitors, cognitive improvement appears short-lived and there are associated toxicity problems.

Given the involvement of oxygen free radicals in the pathogenesis of Alzheimer's disease together with loss of cholinergic function, the compounds of the present invention will afford a novel therapeutic strategy to tackle Alzheimer's disease.

Therefore, the invention provides a compound comprising an antioxidant, preferably a compound of general formula I, chemically joined to choline and/or histidine for use in the treatment of Alzheimer's disease.

The invention also provides the use of a compound comprising an antioxidant, preferably a compound of general formula I chemically joined to choline and/or histidine in the preparation of an agent for the treatment of Alzheimer's disease.

The compounds of the invention may be administered as the free bases or, if more appropriate, as physiologically compatible salts such as hydrochlorides.

The compounds may be used alone but will preferably be admixed with suitable excipients to form a pharmaceutical composition. Therefore, the invention provides in a further aspect a pharmaceutical or veterinary composition comprising a compound according to the present invention together with a pharmacologically or veterinarily acceptable excipient.

The compositions may be adapted for administration by any suitable route, for example by oral, nasal, buccal or transdermal administration or by parenteral, for example intravenous or intramuscular administration.

Compositions for oral administration may be formulated as liquids, for example solutions or suspensions which may contain liposomes or micelles, or as solids, for example as tablets or capsules.

Nasal formulations may be in the form of liquids, for example solutions or micellar or liposomal compositions, which can be administered as a spray and buccal and transdermal formulations may be formulated as solid slow release compositions.

Compositions adapted for parenteral administration will generally be in the form of injectable solutions, again, these may, if necessary, have the compounds contained in micelles or liposomes.

All of these types of composition are well known and could readily be prepared by those skilled in the art.

The dosage of the compound will depend very much upon the condition to be treated and the particular antioxidant and precursor from which the compound is constructed. However, as a general guide, the dosage fo the compounds of the present invention may be from 1 to 500 mg/kg/day.

The invention will now be described in greater detail by reference to the following non limiting examples.

Example 1: Synthesis of 6-hydroxy-2,2,5,7,8-pentamethylbenzopyran (An Antioxidant).

Method 1

Trimethyl quinone (6.15g, 0.39 mol) and freshly fused zinc chloride (5.24g, 0.39mol) were dissolved in acetic acid (30ml) under argon with constant stirring. The solution was refluxed at 100°C (3 hrs), following which isoprene (2.78g, 0.4 mol) was added

dropwise. The reaction was monitored by thin layer chromatography (TLC) (ethyl acetate/30-40° petroleum ether, 10:90) for the formation of the product. After completion of the reaction, the solution was poured into ice, neutralised with 5% w/v sodium bicarbonate (300ml)and extracted with ethyl acetate. The organic layer was dried with magnesium sulphate. Evaporation of the solvent gave a brown oil. To this was added methanol (40 ml) and concentrated hydrochloric acid (10 ml)to remove the acetate and the solution was refluxed for 1 hour at 100°C. The methanol was removed under *vacuo*, the solution was neutralised with 5%w/v sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with water and dried with magnesium sulphate. Evaporation of the solvent gave a dark brown oil which was crystallised from 40-60° petroleum ether to afford a light brown crystalline solid product (4.06g, 38%), mp 82-84°C. TLC (ethyl acetate / 40-60° petroleum ether, 10:90) : $R_f = 0.56$.

IR (KBr): ν = 3251 (O-H), 2981-2929 (C-H), 1366 (d, geminal CH_3), 1224 (C-O-C), 1168 (C-O) cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.28 (3H, s, C_2-CH_3), 1.81 (2H, t, C_3-CH_2), 2.10 (6H, s, aromatic CH_3, 2.15 (3H, s, C_8-CH_3), 2.61 (2H, t, C_4-CH_2), 4.22 (1H, s, C_6-OH), 7.22 ($CDCl_3$-d).

^{13}C NMR ($CDCl_3$): δ = 11.30 (c_{5a}, CH_3), 11.81 (c_{8b}, CH_3), 12.24 (c_{7a}, CH_3), 21.12 (C_3,CH_2), 26.7 ((c_{2a},c_{2b}, $2CH_3$), 33.11 (C_4, CH_2), 72.51 (C_2), 117.17 (C_{4a}), 118.6 (C_5), 121.13 (C_7), 122.62 (C_8), 144.63 (C_6), 145.74 (C_{8a}).

MS : M^+ at 220.

Method 2

Trimethylhydroquinone (7.66g, 0.05 mol) and trifluoroacetic acid (30 ml) were mixed together under an atmosphere of argon. 2-methyl-3-buten-2-Official Letter was added slowly over a period of 2 hours. The reaction was monitored by TLC (ethyl acetate/40-60° petroleum ether 10:90). After completion of the reaction, the solution was poured onto ice, neutralised with 5% w/v sodium bicarbonate and washed with water. The organic layer was dried with magnesium sulphate and solvent was removed under *vacuo*. To the resulting oil was added methanol (100 ml), concentrated hydrochloric acid (10 ml) to remove the trifluoroacetates, and the solution was refluxed at 100°C for 1 hour. The solution was then neutralised with 5% w/v sodium bicarbonate, extracted with ethyl acetate, washed with water and the organic layer dried with magnesium sulphate and the solvent was removed under *vacuo* to afford a red oil which on standing in 40-60° petroleum ether afforded the product as a light brown solid (3.20 g, 29%).

Method 3

Trimethylhydroquinone (12.61 g, 0.08mol) was dissolved in trifluoroacetic acid at 100°C under an atmosphere of argon. 3-Methyl-2-buten-1-Official Letter (6.89g, 0.08mol) was added slowly over a period of 3 hours. After completion of the reaction, the reaction mixture was poured onto ice. The organic layer was extracted with ethyl acetate (250ml), neutralised with 5% w/v sodium bicarbonate and washed with water (100 ml x 2). The ethyl acetate was removed under reduced pressure. To the organic layer, methanol (100ml) and concentrated hydrochloric acid (5ml) were added and the solution refluxed for 1 hour. The methanol was removed under reduced pressure. The solution was neutralised with 5% w/v sodium bicarbonate, extracted with ethyl acetate (150 ml) washed with water (100ml) and the solvent removed under *vacuo*. On standing the brown oil solidified. Crystallisation from 40-60° petroleum ether afforded the product as a light brown solid.

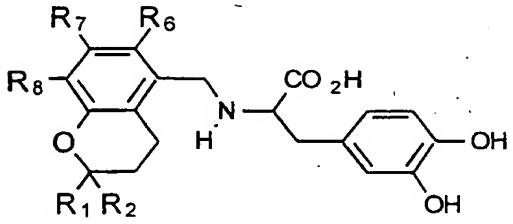
Using analogous methods, the following compounds were made:

2,2,5,7,8-pentamethylbenzopyran from 2,3,5-trimethylphenol and isoprene (Method 1) or 2,3,5-trimethylphenol and 2-methyl-3-buten-2-ol (Method 2);
6-chloro-2,2,-dimethylbenzopyran from p-chlorophenol and isoprene (Method 1);
7,8-dihydroxy-2,2-dimethylbenzopyran from pyrogallol and isoprene (Method 1);
1,2-dihydro-3,3-dimethyl-3H-naphtho-[2,1-b]-pyran from 2-naphthol and isoprene (Method 1);
6-hydroxy-2,2,-dimethylbenzopyran from hydroquinone and isoprene (Method 1);
5-hydroxy-2,2,4,6,7-pentamethylbenzofuran from trimethylhydroquinone and 2-methyl-2-propen-1-ol (Method 2); and
2,2-dimethylbenzofuran from 2,3,5-trimethylphenol and 2-methyl-2-propen-1-ol (Method 2).

A similar reaction was carried out between trimethyl hydroquinone, trimethyl orthoformate and methyl vinyl ketone in methanol to yield 6-hydroxy-2-methoxy-2,5,7,8-trimethylbenzopyran.

A further similar reaction was carried out between trimethylhydroquinone and 2-methyl-2,4-pentanediol in glacial acetic acid to yield 6-hydroxy-2,2,4,5,7,8-hexamethylbenzopyran.

Example 2: Preparation of α-tocopherol-L-DOPA adduct



a) Bromination of α-Tocopherol (Vitamin E).

A solution of bromine (0.235g, 1.47 mmol) in dry chloroform was rapidly mixed with a solution of α-tocopherol (0.500, 1.16 mmol) in chloroform (200 ml) at 0°C. The resulting yellow mixture was allowed to stir for 2 hours. The fumes of HBr evolved were detected using universal indicator paper (pH 2) and were conveniently removed from the reaction mixture using a water aspirator (22 mm Hg). The mixture was purified by washing three times with $NaHCO_3$ (5%, 30 ml) drying with magnesium sulphate and evaporation under reduced pressure to yield an unstable light brown oil. TLC (10% ethyl acetate/ diethyl ether: 1:9, R_f = 0.83).

b) Preparation of L-DOPA Adduct

DOPA can be in racemic form or their individual isomers. The preferred form of the DOPA constituent is L-3,4-dihydroxyphenyl-alanine (chemical registry no: [59-92-7]. The method of synthesising DOPA was that of Yamada *et al. Chem. Pharmaceutical. Bull.* **10**, 693 (1962).

L-3,4-dihydroxyphenylalanine i.e. L-DOPA (1.972 g, 0.01 mole) and freshly prepared 5-bromo-Vitamin E (see example 2, 5.0961g, 0.01 mole) were dissolved in anhydrous N-methyl-pyrrolidin-2-one (250ml) under argon with constant stirring and protected from light. The solution was refluxed at 180°C (24 hrs). The reaction was monitored by

thin layer chromatography (TLC) on silica (Methanol/butanone/conc. Ammonia, 50:40:10) or alumina Chloroform/ methanol, 5:1). After completion of reaction the dark solution was poured onto 1 kg of cracked ice to which 2.5 litres of ethyl acetate were added, and the mixture was adjusted to neutrality using 2M aqueous ammonia. The upper oil layer was removed and concentrated in vacuum to yield an oil.

This residual oil was separated using a medium pressure liquid chromatography apparatus (MPLC) using silica (1 kg) eluted with $CHCl_3$/MeOH 10:1 under gradient conditions finishing with pure methanol). Combining fractions gave a poorly soluble orange oil that was analysed using NMR spectroscopy. The spectra were sensitive to the exact concentration used perhaps due to the formation of aggregates or micelles.

NMR: broadened ^1H NMR (DMSO-D_6,) d 0.82-1.78 (m, 21 H, 1'-12'-H), 0.85 (s, 6H, 4'a- and 8'a-CH_3), 0. 88 (s, 6H, 12'a- and 13' CH_3), 1.32 (s, 3H, 2a-CH_3), 1.5 (singlet, 2H), 1.90 (octet, 2H, J = 6.8 Hz, 3-CH2), 2.15 (s, broad, 6H, aromatic CH_3) 2.51 (2H singlet), 2.7 (1H), 2.74 (t, 2H, J = 6.8 Hz, 4-CH_2), 2.76 (quartet), 2.93 (1H), 4.62 (s, 1H, 6-OH, D_2O exchange)), 3.5 (s, broad 2H, 5-CH_2), 6.5 (complex multiplet, 1H aromatic H), 6.8 complex multiplet, 2H aromatic H), 8.37 (broad 3H). C38H5906N = 625 Daltons. In mass spectrometry, (chemical ionisation mode: CI), the oil gave a weak mass ion in ammonia-CI-MS at 626 Daltons (M+ 1 ion).

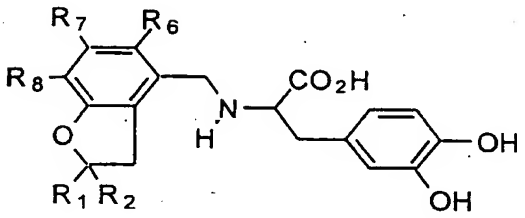
In a second series the products formed from brominated benzofurans and DOPA are attached directly at a position ortho to the phenolic function but could also be attached at any other position including R^7 and R^8. In these series, the following compounds have been prepared:

$R^1 = CH_3$, $R^2 = CH_3$;

R^1 = unbranched hydrocarbon chain which includes ethyl, methyl, propyl etc. up to a maximum of 22 carbon atoms in the chain and $R^2 = CH_3$;

R^1 = phytyl (the isomer with the RRR configuration being preferred) and R^2 = CH_3.

Example 3: Benzofuran-L-DOPA adduct



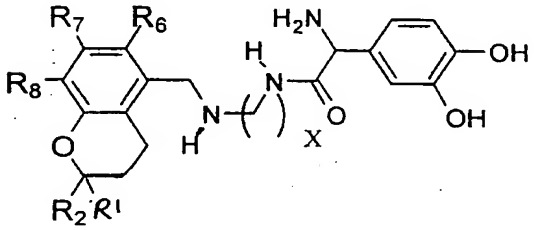
a) 5-bromomethyl-2,3-Dihydro-6-hydroxy-2,2,6,7-tetramethyl-benzofuran (2.85g, 0.01mol) was prepared by reacting 2,3-dihydro-5-hydroxy-2,2,4,6,7-pentamethylbenzofuran (2.06g, 0.01 mol) with one mole equivalent of bromine (1.60g, 0.01 mol) to give an unstable brown gum (2.75g, 96%) by adapting the method of Goodhue *et al* (*Helv. Chem. Acta.*, (1963), 46, 636-649) for the preparation of which was used immediately in the next step.

b) L-3,4-dihdroxyphenylalanine i.e. L-DOPA (1.972g, 0.01 mole) and freshly prepared 5-bromomethyl-2,3-Dihydro-6-hydroxy 2,2,6,7-tetramethyl-benzofuran (2.85g, 0.01 mol) were dissolved anhydrous N-methyl-pyrrolidin-2-one (250ml) under argon with constant stirring, protected from light. The solution was refluxed at 180°C (18 hrs). The reaction was monitored by thin layer chromatography (TLC) on silica (Methanol/butanone/conc. Ammonia, 50:40:10) or alumina Chloroform/ methanol, 5:1). After completion of reaction the dark solution was poured onto 1 kg of cracked ice to which 1 litre of ethyl acetate was added and the mixture was adjusted to neutrality using 2M aqueous ammonia. The upper oil layer was removed and concentrated in vacuum to give a brown oil 2.40g (50%). The solution was suspended in petroleum ether (b.p. 80-100°C) and saturated with anhydrous HCl gas to give a

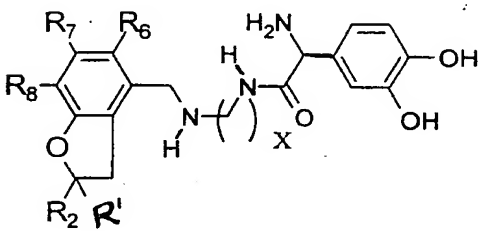
hygroscopic 1.23 g of tan solid which was prone to autoxidation. m.p. >250°C. The compound was stable when stored in a freezer (- 40°C) desiccated under argon.

^{1}H NMR (DMSO-D_6) δ1.47 (s, 6H, alkyl CH_3), 2.15 (s, broad, 6H, aromatic CH_3), 2.5 (s, 2H), 2.7 (q, 1H), 2.8 (s, 2H, CH_2), 2.9 (q, 1H), 3.3 (q, 1H), 6.4(q, 1H, aromatic), 6.8, (2H, dd, Ar-H), ~8.45 (s, v. broad ~4H, Ar-OH exchange with COOH). $C_{22}H_{27}NO_6$ = 401. Compound involatile in EI-MS-direct probe. Weak ion in MS-CI at 402/418.

Example 4: Homologated Antioxidants-DOPA compounds



Benzopyran analogue (n=1)



benzofuran analogue (n = 0)

In a third series, compounds in which the antioxidant and DOPA moiety are separated by aliphatic diamines were constructed. A compound having a carbon bridge with six

carbon atoms was found to have a good solubility profile. However, by changing the length of the linker, different tissues can be targeted since the lipophilicity is changed. The chain length of the linker will then depend on x, where x is the number of carbon atoms between 0 and 22, with x = 0 being the hydrazine analogue. The aliphatic diamine linker chain can also contain heteroatoms, preferably oxygen, nitrogen or sulphur with nitrogen being the preferred heteroatom. Examples of heteroatomic substituted diamines include spermine, spermidine and putrescine. The diamine can also be branched with the preferred substituents being CH_3 alpha to the N atom.

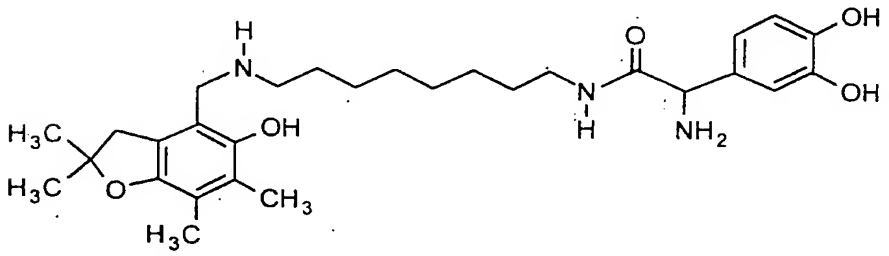
The following compounds were prepared in both the benzopyran and benzofuran series:

In the antioxidant portion, $R^1 = CH_3$, $R^2 = CH_3$;

R^1 = unbranched hydrocarbon chain which includes ethyl, methyl, propyl etc. up to a maximum of 22 carbon atoms in the chain and $R^2 = CH_3$;

R^1 = phytyl (the isomer with the RRR configuration being preferred) and $R^2 = CH_3$.

Antioxidant:hexamethylene diamine:L-DOPA adduct (linked antioxdant).



a) The acid chloride of L-3,4-dihydroxyphenylalanine i.e. L-DOPA (1.972 g, 0.01 mole) was prepared by dissolving it in excess thionyl chloride (1 litre) and refluxing (3 hrs) under anhydrous argon. The excess thionyl chloride was distilled off using a water

pump or high vacuum protected with a KOH guard tube. The oily compound proved unstable and rapidly darkened at room temperature. After preparation, it is best stored desiccated under argon at –40°C and used as soon as possible in the next step.

b) Freshly prepared 5-bromomethyl-2,3-Dihydro-6-hydroxy 2,2,6,7-tetramethyl-benzofuran (2.85 g, 0.01 mol) and pure hexamethylene diamine (1.16, 0.01 mol) were dissolved in anhydrous N-methyl-pyrrolidin-2-one (250ml) under argon with constant stirring, protected from light. The solution was refluxed at 180°C (18 hrs). The intermediate benzopyran-hexamethylenediamine adduct was used without further isolation. The acid chloride of DOPA was warmed (under argon) and added dropwise to the refluxing solution of the benzopyran-hexamethylenediamine adduct using a teflon lined needle. If required, one mole equivalent of triethylamine can be added after all the acid chloride has been added, to accelerate the reaction.

The reaction was monitored by thin layer chromatography (TLC) on silica (Methanol/butanone/conc. Ammonia, 50:40:10) or alumina Chloroform/ methanol, 5:1). After completion of reaction the dark solution was poured onto 2 kg of cracked ice to which 1.5 litre of ethyl acetate was added, and the mixture adjusted to neutrality using 2M aqueous ammonia. The upper oil layer was removed and concentrated in vacuum to give 1.42 g (28%) of a viscous red-brown oil. The solution was suspended in petroleum ether and saturated with anhydrous HCl gas until one mole equivalent had been absorbed by weight, to give a hygrosopic tan solid 1.37 g (25%, monohydrochloride: m.p. >250°C).

^{1}H NMR (DMSO-D_6) δ1.44-1.52 (4H, m), 1.47 (s, 6H, overlapping alkyl CH_3), 1.63-1.77 (4H, m), 2.15 (s, broad, 6H, aromatic CH_3), 2.5 (s, 2H), 2.7 (q, 1H), 2.8 (s, 2H, CH_2), 2.9 (q, 1H), 3.22-3.30 (5H, complex multiplet), 6.4(q, 1H, aromatic), 6.8, (2H, dd, Ar-H), ~8.45 (s, v. broad ~4H, Ar-OH exchange with COOH). $C_{29}H_{43}O_5N_3$ =

513, Compound involatile in EI-MS-direct probe. Electrospray Ions at 514/536 (M +Na).

Examples of other suitable amines are: ethane-1,2-diamine; propane-1,2-diamine; racemic, RR and SS forms of cyclohexane 1,2-diamine; propane 1,3-diamine; butane-1,4-diamine, pentane-1,5-diamine; 2-methylpentane-1,5-diamine; hexane-1,6-diamine; heptane-1,7-diamine; octane-1,8-diamine; nonane-1,9-diamine; decane -1,10-diamine; dodecane-1,12-diamine; putrescine, spermine and spermidine.

In all cases the 5-brominated benzofurans (n = 0) can be replaced by 5-brominatedbenzopyrans as well as 5-bromo-α-tocopherol, or any other lower homologues of Vitamin E provided they contain a methyl grouping on the 5 position: e.g. α and β but not γ or δ. These can all be prepared using the above described method.

In the majority of cases the compounds are either oils, waxes or solids and cannot be recrystallised satisfactorily. It is essential to use column chromatography or preferably MPLC to obtain compounds of pharmaceutical grade.

If desired, the compounds may be converted to their acetates using standard methods: e.g. reacting one mole equivalent of the antioxidant - DOPA conjugate with anhydrous acetic anhydride with 1 millimole equivalent of dimethylaminopyridine or triethylamine at reflux for 24 hours produces the acetates of such compounds, the acetate grouping being found on all three phenolic groups, but not the free amine group. This constitutes the use of protective group that is both biocompatible and known to be removed *in vivo* from compounds such as α-tocopherol.

The free amine can also be protected using a suitable protective grouping including acetate, mesylate, trifluoroacetate and BOC using methods known to practitioners of the art.

In Examples 2 to 4, the DOPA can be replaced by another targeting moiety, for example an amino acid or a dipeptide. Dipeptides may be prepared by standard methods such as dicyclohexylcarbodiimide (DCC) coupling, which is described by Greene and Wuts (, *Protective Groups in Organic Synthesis*, 2nd Edition, Pub. J. Wiley & Sons).

Example 5: Purification

All substances were further purified before biological testing by flash column chromatography or medium pressure liquid chromatography in either normal or reverse phase, separation being carried out under pressurised argon (5psi) on silica gel, Merck 60™ (230-400 mesh) or Laporte Industries Alumina UG1 100 mesh. Vacuum dry column chromatography was carried out in polyethylene tubing either on deactivated silica gel (grade 3) or basic alumina. TLC was carried out on pre-coated silica gel 60 plates in tanks saturated with the indicated solvent. Substances were detected by:

a) spraying with a 5% solution of phosphomolybdic acid in ethanol;
b) observation at 254 nm UV light source;
c) functional group tests;
d) 5% sulphuric acid;
e) 5% aqueous potassium permanganate;
f) iodine tank; or
g) charring with a hot air gun;

as appropriate. Most 4-amino-quinoline compounds are sensitive to reagent (a)and develop intensely coloured blue spots.

Example 6 System for Testing the Efficacy of the Compounds

Low temperature autoxidations (below 100°C) of organic materials (especially hydrocarbons) in the liquid phase and their inhibition by phenols is a well understood process. By the application of rigorous kinetic analysis and rate schemes developed in a well defined chemical system, the absolute and relative rate constants of the autoxidation of biomolecules can be evaluated. The evaluation of absolute rate constants (coefficients) of elementary oxidation reactions is one of the most important steps in kinetic analysis. Comparison of the experimentally derived rate constants with a proposed kinetic reaction scheme can be used to test the proposed models. This allows competing steps which are relatively unimportant to be eliminated from the scheme. The use of this approach clarified the relative and absolute anti-oxidant action of α-tocopherol *in vitro* and demonstrated that it is one of the best chain breaking anti-oxidants so far discovered.

The effects of inhibitors on the autoxidation of various polyunsaturated fatty acids (PUFAs) can be studied in 0.5M sodium dodecyl sulphate (SDS) micelles dissolved in phosphate buffer (pH 7.0) at a temperature of 30°C. A Clarke-type oxygen electrode interfaced to a microcomputer has been used for continuous monitoring of the rate of autoxidation (Ismail *Diss. Abstr. Int. B.*, (1990) **50**, 4539).

The efficiencies of azo-initiators. determined using the induction period method, can be corrected using Surovtsev and Pestov analysis and other new analyses which have been developed during the course of previous investigations. Several different experimental approaches can be used to determine the key parameter τ (the inhibition period). The relative advantages and disadvantages of each approach for determining accurate rates

of initiation (W_i), τ and the absolute rate constants for propagation (k_2), termination ($2k_6$) and inhibition (k_7) in liquid and micellar phase can then be compared.

Quantitative steady state kinetic studies of the order in substrate, RH and the rate of chain initiation show that the classical law:

$$W = k_2[RH]\sqrt{W_i/2k_6}$$

where W is the rate of oxidation;
k_2 is the propagation rate constant of the oxidisable system;
k_6 is the termination rate constant of the oxidisable system;
W_i is the rate of free radical initiation; and
[RH] is the concentration of oxidisable substrate;

applies to benzophenone photo-initiated autoxidation of oleic, linoleic, linolenic and arachidonic acids in SDS. The 'reactivity' ($k_p/2k_t^{1/2}$) for photo-initiated oxidation in SDS micelles was similar to that measured by thermal azo-initiators. These could be used to test both the repair efficiency of antioxidants both in model systems and *in vitro* and *in vivo* test systems, when monitored by chemiluminescence.

Various inhibitors, including a number of phenols, amines and thiols structurally related to α-tocopherol were synthesised in order to determine their structure-antioxidant activity relationships. The rate constant k_7 for abstraction by peroxy radicals of the donatable hydrogen atom from the inhibitors has been previously measured at pH 7 in phosphate buffer at 30°C. The variation in k_7 can then be explained in terms of stereoelectronic and conformational features present in each inhibitor. An amino, thiol or hydroxyl group on the benzenoid moiety, para to the heteroatom is essential for maximal antioxidant activity. The activity decreases in the order $NH_2 \sim OH > HS$. The hypothesis is advanced that the interaction of the p-type

lone pair on the heteroatom stabilises the phenoxyl, aminyl or thiyl radical formed upon abstraction of a hydrogen atom. Contraction of the alicyclic ring from benzopyran to benzofuran results in the p-type orbitals attaining an orthogonal position relative to the plane of the ring, resulting in an increase in the value of k_7.

This method, therefore enables the most efficient anti-oxidant moieties to be selected for combining with the precursor moiety.

Test Results

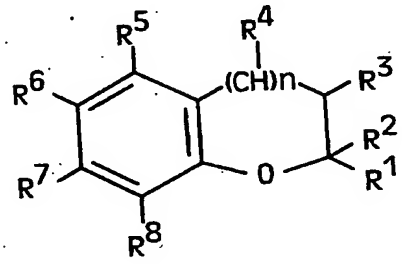
It was found that the initial portion of the kinetic curves illustrating the rate of absorption of oxygen, carried out in the presence of the above antioxidant, are adequately described by standard equations used to test antioxidant efficiency (see equation 230. Ismail thesis, Dissertation abstracts international) Figures 8.1 to 8.22 show representative kinetic curves of the rate of oxygen absorption during the oxidation of linoleic acid (0.257 M in the micellar phase) by DBHN (5.0 x 10-2 M) in the presence of some of the inhibitors listed in table 8.1. Each experiment was repeated at least three times (excluding preliminary investigations), and the average values of k7 are presented in table 8.1. The compound described directly above had a rate constant of 5.31 x 104 (± 32%, average of three runs) when compared to vitamin E as the standard, α-Tocopherol 1.87 x 104 (± 12%, average of 17 runs).

CLAIMS

1. A compound comprising an antioxidant moiety chemically joined to a targeting moiety capable of targeting specific populations of cells by exploiting endogenous uptake systems for biological chemicals.

2. A compound as claimed in claim 1, wherein the antioxidant moiety is either an enzymatic or a non-enzymatic antioxidant which possesses the ability to reduce the formation and/or production of free radical species generated by redox reactions of oxygen and nitrogen occurring in biological systems.

3. A compound as claimed in claim 2, wherein the antioxidant moiety is a compound of general formula I:



(I)

wherein:

R^1 is C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, aryl or heteroaryl, any of which may optionally be substituted with one or more fluoro, chloro, bromo, amino, nitro, azo or diazo groups

Each of R^2, R^4, R^5, R^7 and R^8 is, independently, hydrogen, fluoro, chloro, bromo or C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, any of which may be substituted with one or more fluoro, chloro, bromo, amino, nitro, azo or diazo groups;

R^3 is hydrogen or, together with R^2 or R^4, may form a double bond;

R^6 is OH, NHR^9,SH, Cl, Br or F;

R^9 is C_1-C_6 alkyl, C_2-C_6 alkenyl or C_2-C_6 alkynyl;

n is 0 or 1.

4. A compound as claimed in claim 3, wherein the antioxidant moiety is a compound of general formula I in which R^6 is OH or NH_2 and R^1 is C_2-C_{24} alkenyl optionally substituted by one or more azo or diazo groups, R^5, R^7 and R^8 are methyl or ethyl and n is 1, or a fluorinated derivatives of such a compound.

5. A compound as claimed in claim 1 or claim 2, wherein the antioxidant moiety is:

α-tocopherol;

β-tocopherol;

a derivative of α- or β-tocopherol in which the phytyl group is substituted by one or more azo or diazo groups;

2,2,5,7,8-pentamethylbenzopyran;

6-chloro-2,2,-dimethylbenzopyran;

7,8-dihydroxy-2,2-dimethylbenzopyran;

1,2-dihydro-3,3-dimethyl-3H-naphtho-[2,1-b]-pyran;

6-hydroxy-2,2,-dimethylbenzopyran;

5-hydroxy-2,2,4,6,7-pentamethylbenzofuran;

2,2-dimethylbenzofuran;

6-hydroxy-2-methoxy-2,5,7,8-trimethylbenzopyran;

6-hydroxy-2,2,4,5,7,8-hexamethylbenzopyran;

2,3-Dihydro-6-hydroxy-2,5,7,8,-tetramethyl-2-(tridecyl) benzopyran;

2,3-Dihydro-6-hydroxy-2,5,-dimethyl-2-(4',8',12'-trimethyltridecyl) Naphthopyran;

2,3-Dihydro-6-hydroxy 2,2,5,7,8-pentamethylbenzopyran;

2,3-Dihydro-6-hydroxy 2,2,5,7,-tetramethylbenzopyran;

2,3-Dihydro-5-hydroxy-2,2,4,6,7-pentamethylbenzofuran;

2-Methoxy-2,5,7, 8,teramethylchroman-6-ol;

2,6-Dihydroxy-2,5,7,8-tetramethylbenzopyran;

2-ene-6-hydroxy -2,5,7,8-tetramethylbenzopyran;

6-hydroxy-5,7,8-trimethyl-2-oxo-benzopyran-3-carboxylic acid;
vitamin C;

a protein antioxidant such as superoxide dismutase, catalase or glutathione peroxidase; or

carotene-like substances, for example retinoic acid.

6. A compound as claimed in any one of claims 1 to 5, wherein the targeting moiety is a group which is the precursor of a biological cellular chemical and which can be taken up by specific populations of cells, including neurons.

7. A compound as claimed in any one of claims 1 to 5, wherein the targeting moiety is a transport system amino acid.

8. A compound as claimed in claim 6 or claim 7, wherein the targeting moiety is an amine precursor such as levodopa, 5-hydroxytryptophan, choline or histidine, a polyamine precursor such as spermine, spermidine and putrescine or an amino acid such as glutamate and aspartate.

9. A compound as claimed in any one of claims 1 to 8, wherein the precursor molecule is protected.

10. A compound as claimed in claim 10, wherein the precursor molecule has a protecting group selected from tocopherol, boc, neuropeptides, peptides or α-alkylated amino acid peptides (peptoids).

11. A compound as claimed in any one of claims 1 to 10 wherein the targeting moiety and the anti-oxidant are chemically joined by means of a covalent bond.

12. A compound as claimed in any one of claims 1 to 10, wherein the precursor and the anti-oxidant moieties are joined *via* a linking group.

13. A compound as claimed in claim 12 wherein the linker is -NH-, a methylene chain of from 1 to 50 carbon atoms and optionally containing one or more hetero atoms, which may either interrupt the methylene chain or be at one or both ends of the chain or an aromatic, heteroaromatic, carbocyclic or alicyclic ring having from 5 to 14 atoms and one or more rings.

14. A compound as claimed in claim 13, wherein the linker comprises diamine having from 1 to 20 carbon atoms.

15. A compound as claimed in any one of claims 1 to 14 for use in medicine.

16. A compound as claimed in any one of claims 1 to 14 for use in the treatment of neurodegenerative disorders, including those associated with ageing.

17. The use of a compound as claimed in any one of claims 1 to 14 in the preparation of an agent for the treatment of neurodegenerative disorders, including those associated with ageing.

18. A compound as claimed in any one of claims 1 to 14, in which the precursor moiety is levodopa or a protected form thereof, for use in the treatment of Parkinson's disease.

19. The use of a compound as claimed in any one of claims 1 to 14, in which the precursor moiety is levodopa or a protected form thereof, in the preparation of an agent for the treatment of Parkinson's disease.

20. A compound as claimed in any one of claims 1 to 14, in which the precursor moiety is levodopa or a protected form thereof and/or 5-hydroxytryptophan, for the treatment of neuropathological conditions induced by amphetamines such as MDMA.

21. The use of a compound as claimed in any one of claims 1 to 14, in which the precursor moiety is levodopa or a protected form thereof and/or 5-hydroxytryptophan, in the preparation of an agent for the treatment of neuropathological conditions induced by amphetamines such as MDMA.

22. A compound as claimed in any one of claims 1 to 14, in which the precursor moiety is choline and/or histidine, for use in the treatment of Alzheimer's disease.

23. The use of a compound as claimed in any one of claims 1 to 14, in which the precursor moiety is choline and/or histidine, in the preparation of an agent for the treatment of Alzheimer's disease.

24. A pharmaceutical or veterinary composition comprising a compound as claimed in any one of claims 1 to 14 together with a pharmacologically or veterinarily acceptable excipient.

25. A composition as claimed in claim 24 adapted for administration by any suitable route, for example by the oral, nasal, buccal or transdermal routes or by a parenteral, for example intravenous or intramuscular route.

26. A process for the preparation of a compound as claimed in any one of claims 1 to 14, the process comprising:

a) reacting a brominated antioxidant with a targeting moiety having a free amine group;

b) reacting a halogenated antioxidant with a targeting moiety attached to a linker having a free amine group;

c) reacting a halogenated antioxidant and a halogenated targeting moiety simultaneously with a linker having two free amine groups;

d) reacting a halogenated targeting moiety with an antioxidant having a free amine group; or

e) reacting a targeting moiety attached to a halogenated linker with an antioxidant having a free amine group.

27. The invention substantially as hereinbefore described.